

**Attachment A**

1. (original) A nucleic acid molecule comprising:
  - (A) a first polynucleotide element which encodes an RNA molecule, said RNA molecule comprising:
    - (a) at least one cis-acting sequence element;
    - (b) a first nucleotide sequence comprising a first open reading frame, said first open reading frame having a nucleotide sequence encoding an RNA-dependent RNA polymerase; and
    - (c) at least one second nucleotide sequence selected from the group consisting of:
      - (i) a second open reading frame encoding a polypeptide;
      - (ii) a nucleotide sequence complementary to all or a part of the second open reading frame of (i); and
      - (iii) a nucleotide sequence encoding an untranslated RNA molecule or complement thereof;wherein said second nucleotide sequence is operably linked to a promoter which is recognized by said RNA-dependent RNA polymerase;
  - (B) a second polynucleotide element comprising an origin of replication; and
  - (C) a third polynucleotide element encoding a replication initiation factor capable of recognizing said origin of replication.
2. (original) The nucleic acid molecule of claim 1, wherein said origin of replication is derived from a prokaryotic organism, a eukaryotic organism or a virus.
3. (original) The nucleic acid molecule of claim 2, wherein said origin of replication is derived from a eukaryotic organism selected from the group consisting of yeast, mammals and insects.
4. (original) The nucleic acid molecule of claim 1, wherein said origin of replication is derived from a DNA virus that allows for episomal replication.
5. (original) The nucleic acid molecule of claim 1, wherein said origin of replication is derived from a DNA virus, preferably from a DNA virus selected from the group consisting of Papillomavirus, Polyomavirus, Adenovirus, and Hepadnaviruses.

6. (original) The nucleic acid molecule of claim 1, wherein said origin of replication is derived from a Herpesvirus, and preferably wherein said origin of replication is derived from Epstein-Barr virus (EBV).
7. (original) The nucleic acid molecule of claim 1, wherein said origin of replication is *oriP*.
8. (currently amended) The nucleic acid molecule of ~~any one of the preceding claims~~ claim 1, wherein said replication initiation factor is capable of operating as a plasmid maintenance factor.
9. (currently amended) The nucleic acid molecule of ~~any one of the preceding claims~~ claim 1, wherein said replication initiation factor is derived from a prokaryotic organism, a eukaryotic organism or a virus.
10. (currently amended) The nucleic acid molecule of ~~any one of the preceding claims~~ claim 1, wherein said replication initiation factor is derived from a DNA virus, preferably derived from a DNA virus selected from the group consisting of Herpesvirus, Papillomavirus, Polyomavirus, Adenovirus, and Hepadnavirus.
11. (currently amended) The nucleic acid molecule of ~~any one of the preceding claims~~ claim 1, wherein said replication initiation factor is derived from a Herpesvirus, and preferably wherein said replication initiation factor is derived from EBV.
12. (currently amended) The nucleic acid molecule of ~~any one of the preceding claims~~ claim 1, wherein said replication initiation factor is the EBNA-1 protein or a portion thereof.
13. (currently amended) The nucleic acid molecule of ~~any one of the preceding claims~~ claim 1, wherein said replication initiation factor and said origin of replication are derived from the same organism or the same virus.
14. (currently amended) The nucleic acid molecule of ~~any one of the preceding claims~~ claim 1, wherein said replication initiation factor and said origin of replication are derived from different organisms or viruses.
15. (currently amended) The nucleic acid molecule of ~~any one of the preceding claims~~ claim 1 further comprising a fourth polynucleotide element, wherein said fourth

polynucleotide element comprises a selection marker.

16. (original)The nucleic acid molecule of claim 15, wherein said selection marker confers resistance to Puromycin.
17. (currently amended) The nucleic acid molecule of ~~any one of the preceding claims~~ claim 1, wherein said second open reading frame is in a translatable format after one or more RNAdependent RNA replication events.
18. (currently amended) The nucleic acid molecule of ~~any one of the preceding claims~~ claim 1, wherein said RNA-dependent RNA polymerase is selected from the group consisting of: (a) a temperature-sensitive RNA-dependent RNA polymerase; (b) a noncytopathic RNA-dependent RNA polymerase; and (c) a temperaturesensitive, non-cytopathic RNA-dependent RNA polymerase.
19. (currently amended) The nucleic acid molecule of ~~any one of the preceding claims~~ claim 1, wherein said RNA-dependent RNA polymerase is of viral origin, and preferably wherein said RNA-dependent RNA polymerase is of alphaviral origin.
20. (currently amended) The nucleic acid molecule of ~~any one of the preceding claims~~ claim 1, wherein said RNA-dependent RNA polymerase is derived from a Sindbis virus, a Semliki Forest virus or an Aura virus.
21. (currently amended) The nucleic acid molecule of ~~any one of the preceding claims~~ claim 1, wherein said RNA-dependent RNA polymerase is derived from a virus selected from the group consisting of Bebaru virus, Cabassou virus, Chikungunya virus, Easter equine encephalomyelitis virus, Fort morgan virus, Getah virus, Kyzylagach virus, Mayoaro virus, Middleburg virus, Mucambo virus, Ndumu virus, Pixuna virus, Tonate virus, Trinita virus, Una virus, Western equine encephalomyelitis virus, Whataroa virus, Venezuelan equine encephalomyelitis virus (VEE), and Ross River virus.

22. (original) The nucleic acid molecule of claim 18, wherein said temperature-sensitive RNA-dependent RNA polymerase has replicase activity at temperatures below 34°C which is at least five fold greater than the replicase activity exhibited at 34°C or above.
23. (original) The nucleic acid molecule of claim 18, wherein said temperature-sensitive RNA-dependent RNA polymerase has replicase activity at 34°C which is at least five fold lower than the replicase activity exhibited at 29°C.
24. (currently amended) The nucleic acid molecule of ~~any one of the preceding claims~~ claim 1, wherein said second open reading frame encodes a cytokine, a lymphokine, a tumor necrosis factor, an interferon, a toxic polypeptide, a prodrug or a converting enzyme.
25. (currently amended) The nucleic acid molecule of ~~any one of the preceding claims~~ claim 1, wherein said second nucleotide sequence encodes an untranslated RNA molecule selected from the group consisting of an antisense RNA molecule, a tRNA molecule, a rRNA molecule, and a ribozyme.
26. (original) An expression system comprising:
  - (A) a first polynucleotide element which encodes an RNA molecule, said RNA molecule comprising:
    - (a) at least one cis-acting sequence element;
    - (b) a first nucleotide sequence comprising a first open reading frame, said first open reading frame having a nucleotide sequence encoding an RNA-dependent RNA polymerase; and
    - (c) at least one second nucleotide sequence selected from the group consisting of:
      - (i) a second open reading frame encoding a polypeptide;
      - (ii) a nucleotide sequence complementary to all or a part of the second open reading frame of (i); and
      - (iii) a nucleotide sequence encoding an untranslated RNA molecule or complement thereof;wherein said second nucleotide sequence is operably linked to promoter which is recognized by said RNA-dependent RNA polymerase;

- (B) a second polynucleotide element comprising an origin of replication; and
- (C) a third polynucleotide element encoding a replication initiation factor capable of recognizing said origin of replication.

- 27. (original) The expression system of claim 26, wherein said origin of replication is derived from a prokaryotic organism, a eukaryotic organism or a virus.
- 28. (original) The expression system of claim 27, wherein said origin of replication is derived from an eukaryotic organism selected from the group consisting of yeast, insects and mammals.
- 29. (original) The expression system of claim 26, wherein said origin of replication is derived from a DNA virus that allows for episomal replication.
- 30. (original) The expression system of claim 26, wherein said origin of replication is derived from a DNA virus, preferably from a DNA virus selected from the group consisting of Herpesvirus, Papillomavirus, Polyomavirus, Adenovirus, and Hepadnaviruses.
- 31. (original) The expression system of claim 26, wherein said origin of replication is derived from a Herpesvirus, and preferably wherein said origin of replication is derived from Epstein-Barr virus (EBV).
- 32. (original) The expression system of claim 26, wherein said origin of replication is *oriP*.
- 33. (currently amended) The expression system of ~~any one of the preceding claims~~ claim 1, wherein said replication initiation factor is capable of operating as plasmid maintenance factor.
- 34. (currently amended) The expression system of ~~any one of the preceding claims~~ claim 1, wherein said replication initiation factor is derived from a prokaryotic organism or a eukaryotic organism, or a virus.
- 35. (currently amended) The expression system of ~~any one of the preceding claims~~ claim 1, wherein said replication initiation factor is derived from a DNA virus, preferably derived from a DNA virus selected from the group consisting of Herpesvirus, Papillomavirus, Polyomavirus, Adenovirus, and Hepadnavirus.
- 36. (currently amended) The expression system of ~~any one of the preceding claims~~ claim 1, wherein said replication initiation factor is derived from a Herpesvirus, and preferably wherein said replication initiation factor is derived from EBV.

37. (currently amended) The expression system of ~~any one of the preceding claims~~ claim 1, wherein said replication initiation factor is the EBNA-1 protein, or portion thereof.
38. (currently amended) The expression system of ~~any one of the preceding claims~~ claim 1, wherein said replication initiation factor and said origin of replication are derived from the same organism or the same virus.
39. (currently amended) The expression system of ~~any one of the preceding claims~~ claim 1, wherein said replication initiation factor and said origin of replication are derived from 10 different organisms.
40. (currently amended) The expression system of ~~any one of the preceding claims~~ claim 1, further comprising a fourth polynucleotide element, wherein said fourth polynucleotide element comprises a selection marker.
41. (original) The expression system of claim 40, wherein said selection marker confers resistance to Puromycin.
42. (currently amended) The expression system of ~~any one of the preceding claims~~ claim 1, wherein said second open reading frame is in a translatable format after one or more RNA-dependent RNA replication events.
43. (currently amended) The expression system of ~~any one of the preceding claims~~ claim 1, wherein said first, second and third polynucleotide elements are DNA elements.
44. (currently amended) The expression system of ~~any one of the preceding claims~~ claim 1, wherein said first, second and third polynucleotide elements are each present on a separate nucleic acid molecule.
45. (currently amended) The expression system of ~~any one of the preceding claims~~ claim 1, wherein said first, second and third polynucleotide elements are all present on a single nucleic acid molecule.
46. (currently amended) The expression system of ~~any one of the preceding claims~~ claim 1, wherein said first and said second polynucleotide elements are present on a first nucleic acid molecule, and said third polynucleotide element is present on a second nucleic acid

molecule.

47. (currently amended) The expression system of ~~any one of the preceding claims~~ claim 1 , wherein said second nucleic acid molecule further comprises a fourth polynucleotide element capable of promoting the replication of said second nucleic acid molecule.
48. (currently amended) The expression system of ~~any one of the preceding claims~~ claim 1, wherein said second nucleic acid sequence further comprises an origin of replication.
49. (currently amended) The expression system of ~~any one of the preceding claims~~ claim 1, wherein said RNA-dependent RNA polymerase is selected from the group consisting of: (a) a temperature-sensitive RNA-dependent RNA polymerase; (b) a noncytopathic RNA-dependent RNA polymerase; and (c) a temperaturesensitive, non-cytopathic RNA-dependent RNA polymerase.
50. (currently amended) The expression system of ~~any one of the preceding claims~~ claim 1, wherein said RNA-dependent RNA polymerase is of viral origin, and preferably wherein said RNA-dependent RNA polymerase is of alphaviral origin.
51. (currently amended) The expression system of ~~any one of the preceding claims~~ claim 1, wherein said RNA-dependent RNA polymerase is derived from a Sindbis virus, from a Semliki Forest virus or from an Aura virus.
52. (currently amended) The expression system of ~~any one of the preceding claims~~ claim 1, wherein said RNA-dependent RNA polymerase is derived from a virus selected from the group consisting of Bebaru virus, Cabassou virus, Chikungunya virus, Easter equine encephalomyelitis virus, Fort morgan virus, Getah virus, Kyzylagach virus, Mayoaro virus, Middleburg virus, Mucambo virus, Ndumu virus, Pixuna virus, Tonate virus, Trinita virus, Una virus, Western equine encephalomyelitis virus, Whataroa virus, Venezuelan equine encephalomyelitis virus (VEE), and Ross River virus.
53. (original) The expression system of claim 49, wherein said temperature-sensitive RNA-dependent RNA polymerase has replicase activity at temperatures below 34°C which is at least five fold greater than the replicase activity exhibited at 34°C or above.
54. (original) The expression system of claim 49, wherein said temperature-sensitive RNA-dependent RNA polymerase has replicase activity at 34°C which is at least five fold lower than

- the replicase activity exhibited at 29°C.
55. (currently amended) The expression system of ~~any one of the preceding claims~~ claim 1, wherein said second open reading frame encodes a cytokine, a lymphokine, a tumor necrosis factor, an interferon, a toxic protein, a prodrug or a converting enzyme.
56. (currently amended) The expression system of ~~any one of the preceding claims~~ claim 1, wherein said second nucleotide sequence encodes an untranslated RNA molecule selected from the group consisting of an antisense RNA molecule, a tRNA molecule, a rRNA molecule, and a ribozyme.
57. (original) A method of making a recombinant host cell comprising introducing the nucleic acid molecule of claim 1 into a host cell.
58. (original) The method of claim 57, wherein said RNA-dependent RNA polymerase is selected from the group consisting of: (a) a temperature-sensitive RNAdependent RNA polymerase; (b) a non-cytopathic RNA-dependent RNA polymerase; and (c) a temperature-sensitive, non-cytopathic RNA-dependent RNA polymerase.
59. (original) The method of claim 57, wherein said nucleic acid molecule is introduced by way of transfection.
60. (original) A method of making a recombinant host cell comprising introducing the expression system of claim 26 into a host cell.
61. (original) The method of claim 60, wherein said RNA-dependent RNA polymerase is selected from the group consisting of: (a) a temperature-sensitive RNAdependent RNA polymerase; (b) a non-cytopathic RNA-dependent RNA polymerase; and (c) a temperature-sensitive, non-cytopathic RNA-dependent RNA polymerase.
62. (original) The method of claim 60, wherein said expression system is introduced by way of transfection.
63. (original) A recombinant host cell produced by the method of claim 57.
64. (original) A recombinant host cell produced by the method of claim 60.
65. (original) A recombinant host cell comprising:



(A) a first polynucleotide element which encodes an RNA molecule, said RNA molecule comprising:

- (a) at least one cis-acting sequence element;
- (b) a first nucleotide sequence comprising a first open reading frame, said first open reading frame having a nucleotide sequence encoding an RNA-dependent RNA polymerase; and
- (c) at least one second nucleotide sequence selected from the group consisting of:

- (i) a second open reading frame encoding a polypeptide;
- (ii) a nucleotide sequence complementary to all or a part of the open reading frame of (i); and
- (iii) a nucleotide sequence encoding an untranslated RNA molecule or complement thereof;

wherein said second nucleotide sequence is operably linked to a promoter which is recognized by said RNA-dependent RNA polymerase;

(B) a second polynucleotide element comprising an origin of replication; and

(C) a third polynucleotide element encoding a replication initiation factor capable of recognizing said origin of replication.

- 66. (original) The recombinant host cell of claim 65, wherein said third polynucleotide is stably integrated into the genome of said host cell.
- 67. (original) The recombinant host cell of claim 65, wherein said first and said second polynucleotide elements are present on a first nucleic acid molecule, and said third polynucleotide element is present on a second nucleic acid molecule.
- 68. (original) The recombinant host cell of claim 65, wherein said first, second and third polynucleotide elements are provided on the same nucleic acid molecule.
- 69. (original) The recombinant host cell of claim 65, wherein said host cell comprises more than one copy of said third polynucleotide.
- 70. (original) The recombinant host cell of claim 69, wherein at least one copy of said third polynucleotide is stably integrated into the genome of said host cell.

71. (currently amended) The recombinant host cell of ~~any one of the preceding claims~~ claim 1, wherein said second open reading frame is in a translatable format after one or more RNA-dependent RNA replication events.
72. (currently amended) The recombinant host cell of ~~any one of the preceding claims~~ claim 1, wherein said first, second and third polynucleotide elements are DNA elements.
73. (currently amended) The recombinant host cell of ~~any one of the preceding claims~~ claim 1, further comprising a fourth polynucleotide comprising a selection marker, wherein said selection marker confers resistance to Puromycin.
74. (currently amended) The recombinant host cell of ~~any one of the preceding claims~~ claim 1, wherein said second nucleic acid molecule further comprises a fifth polynucleotide element capable of promoting the replication of said second nucleic acid molecule.
75. (currently amended) The recombinant host cell of ~~any one of the preceding claims~~ claim 1, wherein said nucleotide sequence further comprises an origin of replication.
76. (currently amended) The recombinant host cell of ~~any one of the preceding claims~~ claim 1, wherein said RNA-dependent RNA polymerase is selected from the group consisting of: (a) a temperature-sensitive RNA-dependent RNA polymerase; (b) a noncytopathic RNA-dependent RNA polymerase; and (c) a temperaturesensitive, non-cytopathic RNA-dependent RNA polymerase.
77. (currently amended) The recombinant host cell of ~~any one of the preceding claims~~ claim 1, wherein said recombinant host cell is a mammalian cell, and wherein preferably said mammalian cell is selected from the group consisting of a human cell, a primate cell and a rodent cell.
78. (original) A method for producing a polypeptide or untranslated RNA molecule, said method comprising:
  - (a) introducing the nucleic acid molecule of claim 1 into a host cell to produce a recombinant host cell; and
  - (b) culturing said recombinant host cell under conditions suitable for expression of said polypeptide or untranslated RNA molecule.

79. (original) The method of claim 78, further comprising recovering said polypeptide or untranslated RNA molecule.
80. (original) A method for producing a polypeptide or untranslated RNA molecule, said method comprising:
- (a) introducing the expression system of claim 26 into a host cell to produce a recombinant host cell; and
  - (b) culturing said recombinant host cell under conditions suitable for expression of said polypeptide or untranslated RNA molecule.
81. (original) The method of claim 80, further comprising recovering said polypeptide or untranslated RNA molecule.
82. (original) A method for regulating the expression of a polypeptide or an untranslated RNA molecule, said method comprising:
- (a) introducing the nucleic acid molecule of claim 18 into a host cell to produce a recombinant host cell;
  - (b) growing said recombinant host cell under suitable culture conditions; and
  - (c) changing the temperature of the recombinant host cell culture from:
    - (i) a permissive temperature to a restrictive temperature, or
    - (ii) a restrictive temperature to a permissive temperature;
- wherein said polypeptide or untranslated RNA molecule is encoded by said first polynucleotide.
83. (original) The method of claim 82, wherein said polypeptide is a polypeptide that is toxic to said host cell.
84. (original) A method for regulating the expression of a polypeptide or an untranslated RNA molecule, said method comprising:
- (a) introducing the expression system of claim 49 into a host cell to produce a recombinant host cell;
  - (b) growing said recombinant host cell under suitable culture conditions; and
  - (c) changing the temperature of the recombinant host cell culture from:
    - (i) a permissive temperature to a restrictive temperature, or
    - (ii) a restrictive temperature to a permissive temperature;

wherein said polypeptide or untranslated RNA molecule is encoded by said first polynucleotide.

85. (original) The method of claim 84, wherein said polypeptide is a polypeptide that is toxic to said host cell.